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(RESEARCH ARTICLE)

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GARLIC: Nature's potent antifungal and antibacterial ally - cream formulation and evaluation

Pranali Kunteshwar Rabade *, Rajesh Z. Mujariya and Manjeet Singh

Department of Pharmaceutics, Institute of Pharmaceutical Science and Research (IPSR), Balaghat, Madhya Pradesh, 481331, India.

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Abstract

In the pursuit of effective and safe treatments for infectious diseases, herbal remedies have garnered significant attention due to their diverse phytochemical composition and potential therapeutic properties. This study aimed to explore the phytochemical profile and evaluate the antibacterial and antifungal properties of herbal garlic extracts. Traditional natural products continue to be sought after for their bioactive compounds, which offer promising avenues for therapeutic intervention in both human and veterinary medicine. Our investigation not only sheds light on the regional variation of stomach disease occurrence, highlighting the role of dietary factors, but also underscores the importance of herbal preparations in medicinal practice. By combining various plant classes, herbal formulations can exert localized effects upon application, penetrating the skin or mucous membranes to target underlying conditions. The effectiveness of these combined medications may lie in their synergistic mechanisms, offering a comprehensive therapeutic approach. This research contributes valuable insights into the potential of herbal remedies as adjuncts or alternatives to conventional treatments, paving the way for further exploration in infectious disease management and prevention strategies.

Keywords: Antibacterial activity; Antifungal activity; Traditional medicine; Garlic; *A. Sativum*

1. Introduction

Over the past two decades, there has been a rise in the prevalence of fungal infections, marking them as emerging conditions within hospital environment. Among hospital-acquired infections, bacteraemia and fungaemia have become increasingly common. The evolving landscape of immunosuppressive illnesses and related conditions has significantly impacted the epidemiology of fungal infections among hospitalized patients. As a result, the epidemiological dynamics of invasive fungal infections are undergoing a significant transformation, indicating a critical juncture in their understanding and management [1].

Fungal disease This is increasing in very high ratio due to certain reason including opportunistic infection [2,3].

Fungal infection caused by Candida has become more prevalent than Escherichia coli and Pseudomonas sp., Asprgellosis sp. and other sp. [4]. *Candida albicans* is the most common species in the genus which has been implicated in Candidiasis [5].

Candidiasis, an opportunistic infection caused by Candida, can affect in various parts of the body, including the oral cavity, vagina, penis, and other areas. Left untreated, Candida infections leads to the risk of a systemic infection, potentially leading to sepsis and involving multiple organs. Within the oral cavity, Candida species present in various

* Corresponding author: Pranali Kunteshwar Rabade

forms, including C. albicans, C. glabrata, C. krusei, C. parapsilosis, C. pseudotropicalis, C. stellatoidea, and C. tropicalis [6].

The purpose of the current study is also based on the medicinal property of a plant i.e. Garlic (*Allium sativum*) Garlic oil shows a wide range antimicrobial activity.

The key significance of present research is the medicinal value of garlic is its broadspectrum therapeutic effect with minimal toxicity [7].

Garlic, with its leaves, flowers, and cloves, has a rich history as one of the earliest medicinal plants, revered since ancient times for its therapeutic properties in treating various human illnesses. A primary chemical constituent of garlic oil, alliin, exhibits remarkable antimicrobial activity. This oil comprises six sulfur-containing compounds, including allicin, ajoene, diallyl disulfide, dithiin, and S-allyl cysteine, contributing to garlic's distinctive aroma and taste. Diallyl disulfide emerges as a pivotal component, renowned for its potent antibiotic and antifungal properties within garlic [9].

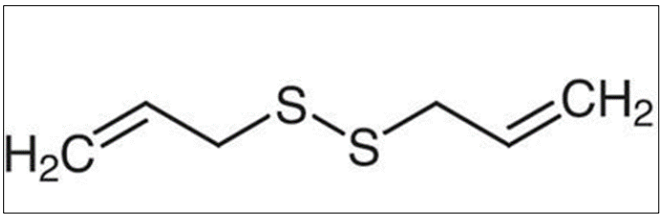


Figure 1 Structure of dially disulfide

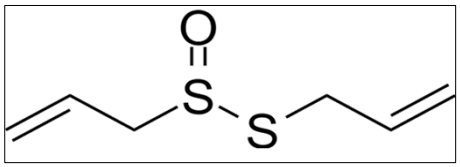


Figure 2 Structure of Allicin

1.1. Dermatologic Application

1.1.1. Antifungal [10]

The research study suggests that compounds like diallyl sulphide and diallyl disulphide found in garlic inhibit enzymes and morphological changes in *Candida albicans*, potentially serving as potent antifungal agents for candidiasis. Additionally, ajoene, derived from garlic, has shown promising results in treating athlete's foot when formulated into a cream, offering a cost-effective and accessible option for managing superficial fungal infections, especially in resource-limited healthcare settings.

1.1.2. Antioxidant effect [11]

The aged garlic extract contains key compounds like S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC) that act as antioxidants, protecting cells from oxidative damage. These antioxidants work by neutralizing harmful reactive oxygen species (ROS), which are known to cause cellular damage and contribute to aging and disease. Additionally, aged garlic extract helps boost the activity of important antioxidant enzymes like superoxide dismutase, catalase, and glutathione peroxidase, further enhancing the cell's ability to combat oxidative stress. Moreover, it increases the levels of glutathione, a powerful antioxidant molecule, within the cells, providing an additional layer of protection against oxidative damage.

1.1.3. UVB Protection [12,13]

Protective benefits against UVB-induced skin damage. UVB radiation from the sun can cause oxidative stress, inflammation, and DNA damage, leading to sunburn, premature aging, and an increased risk of skin cancer. Garlic contains compounds such as allicin and sulfur-containing compounds that possess antioxidant properties, scavenging

free radicals generated by UVB exposure and reducing oxidative stress in the skin. Additionally, garlic's anti-inflammatory effects may help alleviate UVB-induced inflammation and redness. While further studies are needed to elucidate the extent of garlic's UVB protection and optimal application methods, incorporating garlic into skincare formulations or diets may offer supplementary protection against the harmful effects of UVB radiation, complementing traditional sun protection measures such as sunscreen use and sun avoidance during peak hours.

1.1.4. Wound healing [14,15]

Research conducted by Bojs *et al.* highlights that allergic reactions to garlic might have a positive impact on wound healing. Furthermore, studies on chicken skin wounds treated with aged garlic extract revealed enhanced re-epithelialization and increased neovascularization in a dose-dependent manner, suggesting potential wound healing benefits. Additionally, garlic components have demonstrated antiviral properties, inhibiting the proliferation of virally infected cells. For instance, a placebo-controlled trial observed complete resolution of cutaneous warts without recurrence following the application of chloroform extracts of garlic over a period of 3–4 months. These findings underscore the multifaceted therapeutic potential of garlic in dermatology, ranging from wound healing to combating viral infections and skin conditions like warts.

1.1.5. Anti-aging

garlic offers notable benefits for the long-term proliferative capacity of fibroblasts, cells crucial for maintaining skin elasticity and structure. This finding implies that garlic may serve as a promising anti-aging and rejuvenating agent. By enhancing the ability of fibroblasts to proliferate over an extended period, garlic potentially supports skin health and vitality, contributing to a more youthful appearance and improved skin texture. Thus, incorporating garlic into skincare routines or diets may offer a natural and effective approach to combatting signs of aging and promoting overall skin rejuvenation [16].

1.2. Mechanism of action

Allicin has short life and shows higher reactivity and importantly it is nonspecific to particular protein. So, the microbial agent like bacteria virus fungi can’t deal with its actual mechanism of action. In mammalian cell lines, allicin induces, cell death and cell proliferation. The increase in plasma membrane permeability, shows growth inhibition of various species of bacteria and fungi. It also reduces biofilm development in a concentration dependent manner. Allicin does not induce leakage, fusion or aggregation of cell membrane. The high permeability of allicin through membranes may greatly enhanced by intracellular interactions with thiols. And because of this various kind of mechanism of action, it could be a key to sustainable drug design and leads to emergence of multidrug resistant bacterial strains. It acts on both gram +ve and gram -ve and resists to bacteria and fungi strains growth by acting as antimicrobial. Single and combinational with other antimicrobial may increase its action. Allicin is volatile in nature and it may kill bacteria through gas phase. One hour exposure of allicin can stop complete microbial growth [17].

1.3. Drug profile

Figure 3 *Allium sativum*

- **Medicinal Species:** *Allium sativum* L.
- **Botanical Family:** Liliaceae
- **Common Names (Synonyms):** Garlic (Eng.), lasun (Hindi), Ransom & Lahsuna (Sanskrit), Knoblauch
- **Geographical Source**: Central Asia, Southern Europe, USA, India
- **Chemical Constituents:** Allicin is an odorless sulfur containing chemical derived from the amino acid cysteine.

When garlic bulbs are crushed, Alliin is converted into another compound called Allicin. Allicin is further broken down to a compound called Ajoene, which may be the substance that inhibits blockage in blood vessels from clots and atherosclerosis.

Components: Diallyl thiosulfonate (allicin)Diallyl Sulfide, (DAS) Diallyl Disulfide (DADS) Diallyl trisulfide (DATS), E/Z-ajoene, s-allyl-cysteine (SAC) and s-allylcysteinesulfoxide (Allin) Allicin (released when crushed) an amino acid which gives Garlic its strong odor and is responsible for the powerful pharmacological properties of the plant.

- Germanium
- Magnesium
- Selenium
- Vitamin A
- Vitamin C
- Volatile oil of which about 0.5% is composed of sulfur-containing compounds.
- Zinc.
- It also contains 65% water, 28% carbohydrate, 2.3% organo sulphur compound, 2% proteins, 1.2% Free amino [8].

1.3.1. Uses

- Antifungal,
- Antibacterial,
- Wound healing,
- Antioxidant property,
- Antiaging.

2. Material and method

2.1. Materials

Propylene glycol, Beeswax, Stearyl alcohol, Cetyl alcohol, Triethanolamine, Propyl paraben, Methyl paraben, Liquid Paraffin, Stearic acid, Peppermint oil, all the solvents used as AR Grade.

Garlic oil is extracted from Garlic respectively by steam distillation in the laboratory.

2.2. Biological source

Garlic is obtained from ripe bulb of *Allium sativum* Linn. Family: Liliaceae. Chemical Constituents: Allicin, Alliin, volatile and fatty oils, mucilage and albumin.

2.3. Collection of sample

The *Allium sativum* L. was collected from Nagarjuna Medicinal plant garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India and authenticated by Dr. D.K. Koche Head of Botany Department by Shri Shivaji Education Society Amravati's Shri Shivaji College of Arts, Commerce and Science, Akola, Maharashtra and notated as identification voucher No. SSC / B-10/ 2024.

2.4. Extraction of Garlic oil

Extraction method is the first step to separate desired natural product from the raw materials.

Oil of garlic extraction via steam distillation using a Clevenger apparatus involves placing crushed garlic cloves and water in a round-bottom flask (RBF). Steam generated from boiling water vaporizes volatile compounds, including garlic oil.

Vapors pass through a condenser, where they condense into a liquid phase. The resulting mixture of water and garlic oil is collected, with oil separated due to its lower density. The oil is then transferred to an airtight container for storage [2].

2.5. Preparation and Development of Cream

2.5.1. Preparation of oil Phase

White Bees Wax, stearic acid, stearyl alcohol, cetyl alcohol were melted in a stainless steel vessel. To this mixture Liquid paraffin were added and allowed to melt. The temperature of oil phase maintained between 65 – 70 °C.

2.5.2. Preparation of Aqueous phase

Water was heated to 65 – 70 °C. In this weighed propylene glycol, triethanolamine, methyl paraben and propyl paraben were added the temperature of the phase was maintained at 65 – 70 °C.

2.5.3. Development of Cream formulation

Total Oil portion was then slowly incorporated into the aqueous phase at 65 – 70 °C and mixed for 10 to 15 Minutes. When the water and oil phase were at the same temperature, the aqueous phase was slowly added to the oil phase with moderate agitation and was kept stirred until the temperature dropped to 40 °C. and garlic oil was added to it. The emulsion was cooled to room temperature to form a semisolid cream base.

pH of cream kept between 4.5 – 6.5.

Preparation of oil phase Final formulation F1 Final formulation F2

Figure 4 Stages of garlic oil cream formulation

2.6. Formulation

2.6.1. Formulation 1 (F1) table 1 of two Phase

Table 1 Part A – Oily Phase

Ingredients	Quality	Activity
Garlic oil	5%	Antifungal
Stearyl alcohol	5%	Emollient
Cetyl alcohol	6.5%	Binding agent
Mineral oil(liquid paraffin)	5%	Moisturizer
Stearic acid	2.5%	Emulsifying agent
White beeswax	1.5%	Thickening agent

Table 2 Part B – Aqueous Phase

Ingredients	Quantity	Activity
Propylene glycol	5%	Humectants
Triethanolamine	2%	Stabilizer
Methyl Paraben	0.01%	Preservative
Propyl paraben	0.04%	Preservative
Distilled water	Upto 100%	Solvent based

2.6.2. Formulation 2 (F2) table 2 of two phase

Table 3 Part – A oily phase

Ingredients	Quality	Activity
Peppermint oil	5%	Antifungal and flavouring agent
Garlic oil	5%	Antifungal
Stearyl alcohol	5%	Emollient
Cetyl alcohol	6.5%	Binding agent
Mineral oil (liquid paraffin)	5%	Moisturizer
Stearic acid	2.5%	Emulsifying agent
White beeswax	1.5%	Thickening agent

Table 4 Part B – Aqueous Phase

Ingredients	Quantity	Activity
Propylene glycol	5%	Humectants
Triethanolamine	2%	Stabilizer
Methyl Paraben	0.01%	Preservative
Propyl paraben	0.04%	Preservative
Distilled water	Upto 100%	Solvent based

2.7. Evaluation

Take about 1 gram of cream in a clean petri dish and observe visually.

2.7.1 Physical examination

The prepared topical creams were inspected visually for their color, homogeneity, consistency, spreadability and phase separation. The pH was measured in each cream, using a pH meter, which was calibrated before each use with standard buffer solutions at pH 4, 7, 9. The electrode was inserted in to the sample 10 min priors to taking the reading at room temperature. The pH of a topical preparation should be within the pH range corresponding to the pH of the skin, namely, 4.5 - 6.5.

2.7.1. Viscosity

The viscosity of the formulated creams was assessed using a Brookfield Viscometer LVD equipped with spindle S 94, with measurements conducted at varying speeds and shear rates. Speed settings ranged from 0.10 to 0.50 rpm, with

equilibration periods of 60 seconds between successive speeds. Shear rates ranged from 0.20 s^-1 to 1.0 s^-1. All viscosity determinations were carried out at room temperature.

2.7.2. Tube extrudability

In the present study, the method adopted for evaluating formulation for extrudability was based upon the quantity in percentage cream extruded from tube on application of finger pressure 7. More quantity extruded better was extrudability. The formulation under study was filled in a clean, lacquered aluminium collapsible 5 gm tube with a nasal tip of 5 mm opening and applied the pressure on the tube by the help of finger. Tube extrudability was then determined by measuring the amount of cream extruded through the tip when a pressure was applied on a tube.

2.7.3. Microbiological studies

Topical formulation with broad, non-resistance promoting activity against staphylococci, streptococci, dermatophytes or yeast or molds can be of great use in dermatology preparation were infections are often mixed. Since formulation containing antimicrobial agents as active moiety, it is likely to protect from microbial growth. To determine the activity of formulation is subject to study the prepared formulation with standard method called Disk diffusion method and the inhibition zone diameters were measured with the help of zone reader.

3. Results and discussion

3.1. Physical Evaluation (organoleptic characteristics)

The cream is white, appealing appearance and smooth texture, and they were all homogenous with no signs of phase separation.

3.2. pH measurement

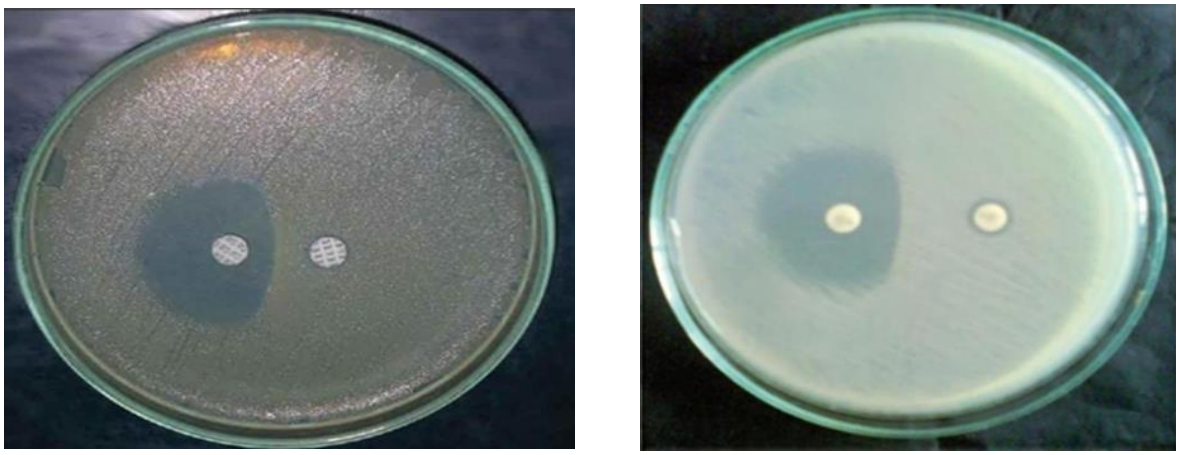
The pH of the cream was found to 6.3. The pH should not be too acidic as it may cause skin irritation and should not be too alkaline as it may cause scaly skin.

3.3. Viscosity measurement

Viscosity was measured by Brookfield viscometer and it was found to be 62570 cps.

3.4. Microbiological studies

From the microbial study it was found that the cream showing good effects on microbial growth and the zone of inhibition was calculated by zone reader. The zone of E.coli it was 33.84 mm.



Against *E. coli* Against *Candida albicans*

Figure 5 Formulated cream showing zone of inhibition

Table 5 Microbial Studies

Bacteria	***Candida albicans***	**E.coli**
Zone of inhibition	41.35 mm	33.84 mm

3.5. Skin irritancy test

As Herbal antifungal cream shows antifungal activity against *Candida albicans* it can be formulated as antifungal formulation (cream).

Skin irritancy testing was conducted to ensure that the herbal antifungal cream formulations did not elicit adverse effects on human skin cells or tissues, such as swelling, redness, or inflammation. A designated area on the left hand dorsal surface was marked, and the cream was applied using a spatula, with the time noted. Irritancy, erythema, and edema were assessed at regular intervals over a period of 24 hours. No significant irritation was observed following application of the herbal antifungal cream, indicating its safety for use.

3.6. Observation

The prepared both formulations (cream) showed good spread ability, no evidence of phase separation and good consistency during the study period. Though stability parameters like visual appearance, is same but the F2 shows better fragrance compare to the formulation F1. And both the formulations showed that there was no significant variation during the study period.

4. Conclusion

The incorporation of herbal and bioactive components in cosmetic cream not only influences the skin's biological functions but also enriches it with vital nutrients, essential for maintaining optimal skin health and combating antifungal infections. Throughout the study period, formulation F2 exhibited remarkable properties including excellent spreadability, absence of phase separation, and consistent texture. However, an initial setback was encountered with formulation F1 due to its unpleasant odor attributed to garlic oil. To overcome this challenge, formulation F2 was devised, incorporating peppermint oil to enhance the preparation while effectively masking the garlic odor. Additionally, peppermint oil served as a tertiary antifungal agent. The integration of antimicrobial agents with garlic oil further bolstered the cream's diffusion rate and antibacterial efficacy. Rigorous chemical and physical tests confirmed the cream's suitability for topical application, rendering it effective in safeguarding against skin infections caused by fungi or bacteria.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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